Two Greek siblings with sepiapterin reductase deficiency

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ABSTRACT

Background: Sepiapterin reductase (SR) deficiency is a rare inherited disorder of neurotransmitter metabolism; less than 25 cases have been described in the literature so far.

Methods: We describe the clinical history and extensive cerebrospinal fluid (CSF) and urine examination of two Greek siblings with the diagnosis of SR deficiency. The diagnosis was confirmed by enzyme activity measurement in cultured fibroblasts and by mutation analysis.

Results: Both patients suffered from a progressive and complex l-dopa responsive movement disorder. Very low concentrations of the neurotransmitter metabolites homovanillic acid (HVA), 5-hydroxyindolacetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylethylenglycol (MHPG) were observed in CSF. CSF neopterin and biopterin concentrations were abnormal in one case only, whereas in both cases sepiapterin concentrations were abnormally high and 5-hydroxytryptophan was undetectable. Urine concentrations of HVA, 5-HIAA and vanillyl mandelic acid (VMA) were decreased in both cases. Both patients had no detectable SR enzyme activity in primary dermal fibroblasts, and upon analysis of genomic DNA revealed the same homozygous point mutation introducing a premature stop codon into the reading frame of the SPR gene (mutant allele K251X).

Conclusions: Our cases illustrate that, apart from HVA and 5-HIAA analysis, the specific quantification of sepiapterin in CSF, rather than neopterin and biopterin alone, is crucial to the final diagnosis of SR deficiency. In addition, urinary concentrations of neurotransmitter metabolites may be abnormal in SR deficiency and may provide an initial indication of SR deficiency before CSF analysis is performed. The known, impressive beneficial response of SR deficient patients to treatment with l-dopa, is illustrated again in our cases.

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Tetrahydrobiopterin (BH4) is an essential co-factor for the enzymes phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), the last two being involved in the biosynthesis of the neurotransmitters serotonin and dopamine (Fig. 1). Besides, BH4 is a co-factor for nitric oxide synthase. BH4 is synthesized in a number of enzymatic reactions, involving GTP cyclohydrolase (GTPCH, E.C. 3.5.4.16), 6-pyruvoyltetrahydropterin (6-PTP) synthase (PTPS, E.C. 4.6.1.10) and sepiapterin reductase (SR, E.C. 1.1.1.153) (Fig. 2) [1].

SR deficiency is a rare condition with currently less than 25 patients described world-wide (as collected in the BIODEF database http://www.bh4.org/BH4Databases.asp).

Patients with SR deficiency generally present with psychomotor retardation, axial hypotonia and spasticity or dystonia of the limbs. Remarkable diurnal fluctuation of the movement disorder can be found [1–5]. Other neurological signs and symptoms may include microcephaly, tremor, seizures, oculogyric crises and hyper salivation [1–3,5]. Hypersomnolence has been described in a single case [6].

The diagnosis of SR deficiency relies on the examination of cerebrospinal fluid (CSF) which shows very low levels of the neurotransmitter metabolites homovanillic acid (HVA), 5-hydroxyindolacetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylethylenglycol (MHPG). This pattern is also observed in patients suffering from aromatic amino acid decarboxylase (AADC) deficiency [7], another rare disorder of neurotransmitter biosynthesis. In contrast to the findings in AADC deficiency, CSF concentrations of 5-hydroxytryptophan (5-HTP), l-dopa and 3-methoxy-tyrosine are not elevated in SR deficiency. Furthermore, in AADC deficiency, urinary concentra-
tions of vanillactic acid are increased while this compound is not elevated in SR deficiency. Ultimately, the defect in BH4 biosynthesis caused by SR deficiency is demonstrated at the metabolite level by CSF analysis, which reveals increased concentrations of total biopterin, 7,8-dihydrobiopterin (BH2) and, specifically, sepiapterin [2–4,8]. The diagnosis can be confirmed at the enzymatic level in fibroblasts or erythrocytes[1] and by mutation analysis of the SPR gene [9]. Seven different disease-causing mutations are known, including missense mutations and deletions and substitutions of nucleotides leading to amino acid substitutions, frame shifts or a truncated protein [9].

SR deficiency does not lead to elevated blood levels of phenylalanine and is therefore not detected in neonatal screening. The absence of hyperphenylalaninemia in SR deficiency is due to the existence of two alternative pathways of BH4 synthesis in peripheral organs (Fig. 2). In the absence of SR activity, 6-PTP is first converted into 1-OXPH4 by the enzymes aldose reductase (AR, AKR1B1, E.C. 1.1.1.21) and carbonyl reductase (CR, E.C. 1.1.1.184), and subsequently non-enzymatically converted into sepiapterin. Finally, via CR and a peripheral dihydrofolate reductase (DHFR) sepiapterin is converted into BH4 [4,10]. Alternatively, 6-PTP can be reduced by the aldo-keto reductase AKR1C3 (E.C. 1.1.1.213) to 1’-hydroxy-2’-oxo-propylterahydropterin (2-OXPH4), which is further converted to BH4 [11].

Patients with SR deficiency clinically respond very well to treatment with L-dopa/Carbidopa and 5-HTP, which may also lead to normalization of CSF neurotransmitter levels [3,4]. Long-term treatment with L-dopa is usually well-tolerated and leads to sustained clinical and biochemical improvement [3].

In this manuscript we describe two Greek siblings with delayed psychomotor development and a complex movement disorder, diagnosed with SR deficiency after CSF analysis. The analysis of increased CSF concentrations of sepiapterin appeared essential for the diagnosis. This report highlights the importance of detailed CSF analysis to diagnose a neurotransmitter disease in patients with early motor impairment.

Methods
Neurotransmitter metabolite analysis in CSF

Because the concentrations of HVA and 5-HIAA vary in the different fractions of CSF, we used the 6th–8th milliliter to analyze these neurotransmitter metabolites. HVA, 5-HIAA, MHPG, 6-dopa, 3-methoxy-tirosine and 5-HTP were analyzed as follows. CSF was prediluted three times in 0.03% formic acid and 150 µl was directly injected. High performance liquid chromatography (HPLC) was carried out using a mobile phase of 0.02 M sodium acetate, 0.3 mM sodium EDTA, 50 mM NaCl and 2.5% methanol (pH 4.15) on an Atlantis Tm column (150 mm; internal diameter 4.6 mm, Waters, Milford, MA). Detection was performed by a passing the eluate through a serially connected coulometric electrochemical detector (E1: 0 mV; E2: 425 mV; type Esa Coulochem II, Interscience; Chelmsford, MA) and fluorimeter (excitation wavelength, 278 nm; emission wavelength, 325 nm; type Waters 474). The use of the fluorimeter allows the analysis of 6-dopa, 3-methoxy-tirosine and 5-HTP together with HVA, 5-HIAA and MHPG within reasonable run times. Furthermore, in this way the concentrations of HVA, 5-HIAA and MHPG are double-checked and can be corrected for confounding components that co-elute with one of these peaks either and that are detected by the electrochemical detector. Data were processed using PC1000 software (TSP, San Jose, CA). The results of this methodology were similar to a previously described method [12].
Analysis of pterins in CSF

Directly after withdrawal, 1 ml of CSF is added to a tube containing 1 mg dithiotreitol and 33 mg trichloroacetic acid. Contrary to the analysis of neurotransmitter metabolites, the concentration of neopterin and biopterin in CSF is not dependent on the fraction (our unpublished findings). The tube is protected against light and stored at $-80^\circ$C until analysis. The CSF samples (0.2 ml) are then mixed with 0.1 ml HCl (0.1 M), 0.1 ml of 2% I$_2$/4% KI solution to oxidize the pterins for 30 min. By using this oxidation procedure all biopterin species in CSF (including BH$_4$, BH$_2$, and B) will be collectively measured as biopterin. Then 0.1 ml ascorbic acid (1%) is added. About 250 l of this final solution is injected into the HPLC. Samples are separated over an UltraPure Torsic Acid column (250 mm, internal diameter 4.6 mm; Screening Devices, Amersfoort, The Netherlands) and pterins are detected by using a fluorescent detector (Jasco 2020-FP, Separation, Hendrik Ido Ambacht, The Netherlands). Detection is performed at the following wavelengths: excitation 350 nm; emission 450 nm.

The mobile phase contains 4.6 g/L NH$_4$HPO$_4$ (pH 3.5). The inter-assay CV for neopterin is 4.5% (at 52 nM, $n = 8$) and for biopterin is 5.6% (at 110 nM, $n = 8$). The intra-assay CV for neopterin is 1.6% (at 34 nM, $n = 6$) and for biopterin is 3.0% (at 10 nM, $n = 6$). Detection limit is 0.21 nM for neopterin and 0.65 nM for biopterin. Sepiapterin was analyzed in an assay separate from the analysis of neopterin and biopterin, essentially as described previously [8]. In brief, CSF is diluted in the mobile phase and separated by using the same HPLC system as used for the detection of neopterin and biopterin. Detection of sepiapterin is performed at the following wave lengths: excitation 425 nm; emission 530 nm. Detection limit is 0.5 nM.

Urine analysis

VMA analysis: 0.5 ml urine is mixed with 0.25 ml citric acid (2 M) and 2 ml ethyl acetate, vortexed and centrifuged. After addition of one milliliter of Na$_2$HPO$_4$ (0.1 M, pH 8.5) to the supernatant, the sample is vortexed and centrifuged again. The water phase is diluted three times with 1 M perchloric acid and injected on an Atlantis Tm column (Waters). Mobile phase consists of 10 mM ammonium acetate, 50 mM NaCl, 0.3 mM NaEDTA and 2.5% v.v. methanol. Detection is performed by using a Jasco 1520 fluorimeter (excitation 278 nm; emission 325 nm).

Noradrenalin, adrenaline and dopamine analysis is performed by using a commercial assay (catalogue number 195–5841, Bio-Rad, Veenendaal, The Netherlands), according to the manufacturer’s instructions. Samples are separated on an Allsphere ODS(2) column (250 mm, internal diameter 4.6 mm; Alltech). Detection is performed by using a Jasco 2020-FP fluorimeter (excitation 278 nm; emission 325 nm).

HVA and 5-HIAA analysis: 200 l urine is mixed with 5 ml water and 20 l 6.6 M formic acid. A Sephadex G-10 column that is subsequently equilibrated with 3 ml 26.7 mM NH$_4$OH and 14 mM formic acid is loaded with 500 l sample. The column is then washed with 3 ml 14 mM formic acid followed by 1.5 ml 5.6 mM Na$_2$HPO$_4$ and eluted with 2.0 ml 26.7 mM NH$_4$OH. The eluate is mixed with 50 l 6.6 M formic acid and 50 l 3.4 M ascorbic acid. Samples are separated on an Hypersil ODS(1) column (250 mm, internal diameter 4.6 mm; Alltech). The mobile phase consists of 42 mM NH$_4$Ac, 0.3 mM NaEDTA, 50 mM NaCl and 0.1125% methanol (pH3.5). Detection is performed by using an Intro electrochemical detector (Antec, Leiden, The Netherlands).
Neopterin and bioperin analysis. Urine samples are protected against light. To 100 μl urine 900 μl 0.1 M HCl is added and 0.1 ml of 2% I2/4% KI solution to oxidize the amines for 60 min. By using this oxidation procedure all bioperin species in CSF (including BH4, BH2, and B) will be collectively measured as bioperin. Then 0.1 ml ascorbic acid (10%) is added. 250 μl of this final solution is analyses by HPLC as described above.

**Sepiapterin reductase activity in fibroblasts**

Sepiapterin reductase activity was determined in unstimulated fibroblasts as described previously [1].

**DNA mutation analysis**

Mutation analysis of the SPR gene was performed in genomic DNA isolated from blood samples from both patients and their parents according to previously described methods [1].

**Patients**

Patient 1 is a 10-year-old female born to non-consanguineous Greek parents, referred to the Department of Pediatrics at the age of 17 months with delayed motor development and generalized hypotonia. Neurological examination disclosed mild pyramidal tract dysfunction with increased tendon reflexes, clonus and positive Rosollimo and Babinski signs, however without apparent increase in muscle tone. She also demonstrated drooling and horizontal nystagmus, which in rare occasions was also cyclic. Routine hematological and biochemical investigations, electroencephalography, brainstem auditory, visual and somatosensory evoked potentials and metabolic screening including blood ammonia and lactate, serum and urinary amino acids, urine for abnormal excretion of oligosaccharides and mucopolysaccharides, as well as urinary organic acids were all normal. MRI demonstrated mild delay in myelin development. Under physical therapy she could walk independently at the age of 2.2 years. At that age she spoke the first words with no further problems hereafter regarding language development. A repeated MRI at the age of 2.5 years was considered normal with normal myelinisation. At the age of 5 years she started being clumsy with occasional falls; her cognitive development was mildly delayed. At the age of 9 years the neurological examination disclosed ataxia, mild dystonia and pyramidal tract signs (increased tendon reflexes with clonus and positive Babinski and Rossolimo signs) and mild athetoid movements. One month after this visit, she experienced paroxysmal episodes with stiffening of her body and she could neither walk nor sit. Additionally, she simultaneously demonstrated oculogyric crises, but also independent of these episodes. A typical episode lasted around 2–3 h and then subsided with the patient falling asleep, with or without the administration of benzodiazepines. During this episode she had temperature alterations with hypo- or hyperthermia without apparent cause (i.e. infection), which could last from one day until one week. Her dystonia and athetosis, as well the cyclic eye movements demonstrated a diurnal fluctuation. Her sleep was disturbed most nights with short duration and several awakenings each night.

Patient 2 is the younger brother of patient 1 and was first admitted at the age of 5 months for psychomotor retardation. MRI demonstrated mildly delayed myelinisation. Similar to his sister, extensive laboratory (including amino acid analysis) and neurophysiologic investigations revealed no abnormalities. During follow-up his motor milestones were delayed: he walked independently at the age of 2 years, spoke the first words at the age of 18 months and 3 word sentences at the age of 3 years. At the age of 6 years he was readmitted because of a seizure-like episode which never recurred. His EEG was normal. A repeated MRI demonstrated normal myelinisation for his age without abnormal findings. At the age of 7 years episodes of sudden stiffening of the legs and subse-

quenty of the arms became manifest. During these episodes, which lasted 1–2 h, he could not speak clearly and his eyes rolled in all directions. The neurological examination between these episodes demonstrated mild truncal ataxia with athetosis and moderate dystonia. His dystonia as well as the athetoid movements followed a diurnal fluctuation, similar to his sister. He also had temperature alterations with hypo- or hyperthermia without apparent cause (i.e. infection), lasting from 6–48 h. His sleep was disturbed too, with short duration, frequent awakenings and frequent irregular movements. Information of both patients is included in the international database of disorders of BH4 biosynthesis (www.bh4.org).

CSF samples were obtained by lumbar puncture at the age of 9 years (patient 1) and 7 years (patient 2), respectively. Treatment was introduced at the age of 10 years (patient 1) and 7 years (patient 2), respectively. The starting dose of L-dopa was 6.25 mg for either patient (which equals 0.2 mg/kg/d for patient 1 and 0.16 mg/kg/d for patient 2) and this was gradually increased until a final dose of 25 mg L-dopa divided into two daily doses (which equals to 0.75 mg/kg/d for patient 1 and 0.65 mg/kg/d for patient 2). L-dopa was given together with carbidopa at a ratio of 1:5. 5-HTP was not administered to either patient. After one year of follow-up both patients demonstrated a clear-cut improvement. Stiffening and ocular deviation disappeared in patient 1, while there was a definite improvement in dystonic posture, athetotic movements and extra-pyramidal syndromes. Stiffening, dystonic posture and ocular deviation even disappeared completely in patient 2, while there was a definite improvement in ataxia and athetosis. Neither of the patients demonstrated evidence of drug-induced dyskinesia either during gradual dose increase or after reaching the end-dose of L-dopa. Both patients were attending normal school with average for their age performances. According to teachers’ informal (phone interview) as well as formal report (official grades) both definitely performed better at school after 6 months of treatment (better attention span and short-time memory, less hyperactivity).

**Results**

Results of the biochemical investigations are summarized in Table 1. In both patients CSF analysis revealed strongly increased concentrations of the catabolic end products of the dopamine (HVA, MHPG) and serotonin pathways (5-HIAA). In addition, the concentrations of the precursor molecules 5-HTP and L-dopa were undetectable, whereas normal concentrations of the 5-l-dopa catabolic 3-methoxy-tyrosine were observed. In CSF of patient 1, slightly elevated concentrations of both neopterin and bioperin were observed, whereas these were normal in CSF of patient 2. In both patients, however, the CSF concentrations of sepiapterin were increased, with a most pronounced increase in patient 1.

Urine analysis demonstrated decreased concentrations of HVA, 5-HIAA and VMA in both patients. Noradrenaline and adrenaline concentrations were normal in both patients, whereas dopamine was slightly decreased in patient 1 only. Serum prolactin was markedly increased in both patients 70.5 ng/ml (patient 1) and 62.3 ng/ml (patient 2) (reference range 2–15 ng/ml), before initiation of therapy, and decreased to 22 and 26 ng/ml, respectively, after 1 year of L-dopa therapy.

The diagnosis of sepiapterin reductase deficiency was confirmed by the absence of enzyme activity in fibroblasts of patient 1. Furthermore, both patients were homozygous for an A>T mutation at base pair 751 in codon 251 (c.751A>T; based on the cdNA sequence M76231.1, and numbering with 1 at the ATG-start codon [9]), resulting in an early stop codon (p.K251X). Heterozygosity for this mutation was confirmed in both parents.
cies revealed increased B and BH2 [6] and low-normal BH4 [3,6] in the specific measurement of each of the native reduced pterin species may yield diagnostic information that is not detected by the measurement of total biopterin.

We observed slightly increased CSF neopterin in one case and normal levels in the other, which is in line with variable concentrations observed in other patients [1,3,4,6]. Given the normal total biopterin and neopterin levels in patient 2, the clue to the diagnosis of SR deficiency was the increased concentration of sepiapterin in the CSF. Clearly increased sepiapterin concentrations in SR deficient patients have only been described in two other cases [3,6]. In other patients the diagnosis was established by reduced SR activity in cultured fibroblasts [1,6]. An interesting novel observation is the complete absence of 5-HTP in the CSF of both patients, which is caused by a strongly reduced conversion of tryptophan into 5-HTP, mediated by TPH requiring BH4 as a co-factor (Fig. 1). This observation is, however, not of diagnostic value.

Urine neopterin was normal and in line with previous reports [1,6]. Total urine biopterin was decreased in one patient, an observation that has been described once [6]. In addition, we described decreased HVA, 5-HIAA and VMA concentrations in urine in both patients. This observation has not been described previously and suggests that urine analysis of neurotransmitter metabolites might be of value in the diagnosis of SR deficiency. This finding requires confirmation in other patients, however. Yet, extensive CSF analysis of pterins and neurotransmitter metabolites, enzyme activity analysis in fibroblasts or mutation analysis is currently still essential to confirm the diagnosis of SR deficiency.

The normal route of BH4 synthesis (Fig. 2) from 6-PTP via 2′-OXPH4 and 1′-OXPH4 is catalyzed by SR. In the absence of SR, two alternative pathways become important. The first identified (“salvage”) pathway involves the conversion of 6-PTP into 2′-OXPH4 by the enzymes aldo reductase (AR or AKR1B1) and carbonyl reductase (CR) (Fig. 2) [11,14]. AR and CR are expressed in the brain at high levels [15,16]. 1′-OXPH4, in turn, is non-enzymatically converted into sepiapterin which is subsequently converted into 7,8-dihydrobiopterin by CR. This latter product can, however, only be converted in peripheral organs into BH4 by the action of dihydrofolate reductase (DHFR), which is very low in the brain [17]. A more recently identified pathway leading to BH4 production involves the conversion of 6-PTP into 2′-OXPH4 by AKR1C3 and the subsequent conversion into BH4 by AR [11,18].

SR deficiency is a disease of the central nervous system, where subnormal levels of BH4 are synthesized, because of the low cerebral expression of AKR1C3 [19]. The salvage pathway does not lead to cerebral production of BH4 because of the lack of DHFR expression. In contrast, however, in peripheral organs the salvage path-

<table>
<thead>
<tr>
<th>Case (age)</th>
<th>Patient 1 (9 years)</th>
<th>Patient 2 (6 years)</th>
<th>Reference range (9 years)</th>
<th>Reference range (6 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF neurotransmitter metabolites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HVA (nM)</td>
<td>55</td>
<td>62</td>
<td>330–668</td>
<td>346–716</td>
</tr>
<tr>
<td>5-HIAA (nM)</td>
<td>5</td>
<td>4</td>
<td>109–214</td>
<td>100–245</td>
</tr>
<tr>
<td>MHPG (nM)</td>
<td>14</td>
<td>12</td>
<td>32–68</td>
<td>37–75</td>
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<tr>
<td>5-Hydroxytryptophan (nM)</td>
<td>&lt;2.0</td>
<td>&lt;2.0</td>
<td>2–25</td>
<td>2–25</td>
</tr>
<tr>
<td>3-Methoxytyrosine (nM)</td>
<td>8</td>
<td>13</td>
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<td>&lt;50</td>
</tr>
<tr>
<td>3-Methoxytyrosine (nM)</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;25</td>
<td>&lt;25</td>
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<tr>
<td>CSF pterins</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Bioperin (nM)</td>
<td>47.1</td>
<td>28.1</td>
<td>10–42</td>
<td>10–42</td>
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<tr>
<td>Neopterin (nM)</td>
<td>15.5</td>
<td>8.0</td>
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<td>2.0–14</td>
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<tr>
<td>Sepiapterin (nM)</td>
<td>20</td>
<td>7.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Urine neurotransmitter metabolites</td>
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<td></td>
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<tr>
<td>HVA (μmol/mmol creatinine)</td>
<td>1.6</td>
<td>0.9</td>
<td>2.0–7.0</td>
<td>2.0–10.0</td>
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<tr>
<td>5-HIAA (μmol/mmol creatinine)</td>
<td>0.8</td>
<td>0.7</td>
<td>1.0–5.0</td>
<td>1.0–10.0</td>
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<tr>
<td>VMA (μmol/mmol creatinine)</td>
<td>1.0</td>
<td>0.9</td>
<td>2.0–8.0</td>
<td>2.0–10.0</td>
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<tr>
<td>Adrenaline (μmol/mmol creatinine)</td>
<td>15.7</td>
<td>24.2</td>
<td>1.5–25</td>
<td>1.5–30.0</td>
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<tr>
<td>Noradrenalin (μmol/mmol creatinine)</td>
<td>12.5</td>
<td>13.6</td>
<td>7.0–60</td>
<td>7.0–85</td>
</tr>
<tr>
<td>Dopamine (μmol/mmol creatinine)</td>
<td>48</td>
<td>80</td>
<td>70–700</td>
<td>70–825</td>
</tr>
<tr>
<td>Urine pterins</td>
<td></td>
<td></td>
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<tr>
<td>Bioperin (nmol/mmol creatinine)</td>
<td>691</td>
<td>325</td>
<td>550–1100</td>
<td>550–1100</td>
</tr>
<tr>
<td>Neopterin (nmol/mmol creatinine)</td>
<td>615</td>
<td>471</td>
<td>400–1400</td>
<td>400–1400</td>
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<tr>
<td>Fibroblasts</td>
<td></td>
<td></td>
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<tr>
<td>SR enzyme activity (µl/mg protein)</td>
<td>&lt;5</td>
<td>NP</td>
<td>99–185</td>
<td>NA</td>
</tr>
<tr>
<td>DNA</td>
<td></td>
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</table>

Abbreviations: HVA, homovanillic acid; 5-HIAA, 5-hydroxyindolacetic acid; MHPG, 3-methoxy-4-hydroxyphenylethanoliglycol; VMA, vanillyl mandelic acid; SR, sepiapterin reductase; NA, not applicable; NP, not performed. Abnormal figures are printed bold.
 way leads to BH4 production and, besides, BH4 can be synthesized by AKR1C3 and AR [11]. This explains the (relatively) normal neopterin and bipterin levels in urine, but not, however, the low urinary HVA, 5-HIAA and VMA concentrations in SR deficient patients. The cerebral neurotransmitter deficiency in SR deficient patients thus cannot be solely explained by the low-to-normal BH4 production in the brain [3], but requires an additional pathological mechanism. Because of the relative absence in the brain of DHFR, 7,8-dihydrobiopterin accumulates, which is a competitive inhibitor of TH and TPH [18] leading to reduction of dopamine and serotonin synthesis in the brain. Finally, in the presence of BH4, the neurotoxic peroxynitrite is produced, which may contribute to brain damage [18,20].

The mutations in the SR gene of our two cases have recently been described in a Caucasian patient [3]. Although slightly different from this patient, the patients described here show clear clinical similarities with that case with regard to age of onset, most important presenting symptoms (developmental delay and hypotonia), clinical evolution (progressive extrapyramidal movement disorder) and occurrence of additional features (oculogyric crises), as well as the response to treatment with l-dopa. As shown for our patient 1, this early stop codon is pathogenic since it leads to undetectable SR enzyme activity. Remarkably, in all literature described patients with SR deficiency, the clinical picture is clearly dominated by the motor impairments. Despite the very low CSF 5-HIAA concentrations, reflecting low cerebral serotonin availability, symptoms and signs related to a cerebral serotonin deficiency are less prominent. One case with marked hypersomnolence has been described [6], and in the patient with the same mutation as our patients, a marked diurnal sleepiness was observed [3]. Our patients displayed “sleep disturbances” and deregulation of body temperature which may both be related to a serotonin deficiency. Other behavioral or psychiatric symptoms were not observed.

In conclusion, SR deficiency is reflected in a specific neurochemical CSF pattern that distinguishes this disorder from other rare disorders of neurotransmitter metabolism (Table 2). Our cases illustrate that the specific quantification of sepiapterin in CSF, rather than neopterin and bipterin alone, is crucial to the final diagnosis. Sepiapterin analysis requires a specific methodology, however. Additionally, urinary concentrations of neurotransmitter metabolites may be abnormal in SR deficiency, but confirmation of the diagnosis of SR deficiency, as well as that of other disorders of BH4 biosynthesis such as GTPCH deficiency, requires CSF analysis. Clinical awareness and appropriate biochemical diagnostic work-up in CSF should warrant early recognition and diagnosis of this treatable neurometabolic disorder.

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References


Table 2
Overview of the patterns of CSF neurotransmitter metabolites and urine vanillactic acid in neurotransmitter biosynthesis disorders

<table>
<thead>
<tr>
<th>Deficient enzyme (reference)</th>
<th>CSF metabolites</th>
<th>Urine metabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HVA</td>
<td>5-HIAA</td>
</tr>
<tr>
<td>TH [21]</td>
<td>Low</td>
<td>N</td>
</tr>
<tr>
<td>AADC [7]</td>
<td>Low Low</td>
<td>High</td>
</tr>
<tr>
<td>GTPCH [22]</td>
<td>Low Low N</td>
<td>Low N</td>
</tr>
<tr>
<td>SR</td>
<td>Low N</td>
<td>Low N</td>
</tr>
<tr>
<td>DPHPR [22]</td>
<td>Low Low N</td>
<td>High</td>
</tr>
<tr>
<td>6-PTPS [22]</td>
<td>Low Low N</td>
<td>N</td>
</tr>
</tbody>
</table>

Abbreviations: CSF, cerebrospinal fluid; TH, tyrosine hydroxylase; AADC, aromatic amino acid decarboxylase; GTPCH, guanosine triphosphate cyclohydrolase; SR, sepiapterin reductase; DPHPR, dihydropteridine reductase; 6-PTPS, 6-pyruvoyl-tetrahydropterin synthase; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindolacetic acid; 5-HTP, 5-hydroxytryptophan; VLA, vanillactic acid; N, normal.

* Total bipterin; in SR deficiency high total bipterin caused by accumulation of 7,8-BH2, whereas in DPHPR deficiency quinonoid-BH2 accumulates.

